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Application News

No. G309

Gas Chromatography

Food Metabolomics Using GC-FID

Samples derived from organisms, including food, contain a great number of components. Research and development on comprehensively analyzing them to clarify the effects on samples, which are produced by differences among lots and manufacturing methods, and predicting the characteristics of unknown samples from their component patterns, has been actively pursued in recent years with the focus on the food field. This is commonly referred to as metabolomics, and is one of the most in-demand analytical methods, in conjunction with analysis software such as multivariate analysis and machine learning.

Mass spectrometers such as LC-MS and GC-MS have been indispensable tools for metabolomics. Mass spectrometers have excellent qualitative analysis performance and are extremely powerful in identifying detected components. On the other hand, they are characterized by a susceptibility to accumulation of contamination in the instrument, along with large variation in the quality of data before and after updating the instrument's tuning information, so they are not suited to obtaining a large amount of data over a long period of time. With GC-MS in particular, the common practice is to measure metabolites after their trimethylsilylation (TMS derivatization), but in many cases the intensity of the peaks of the TMS derivatized compounds decreases over time after derivatization, so in order to obtain good measurement results it can be important to complete the analysis as soon as possible after derivatization.

In this paper, we examine whether metabolomics using GC-MS in this way can be accomplished using GC-FID as an alternative, and we found that a number of peaks comparable to those with GC-MS could be detected, and that the intensities of these peaks were more stable than with GC-MS over both hours and days. This stability of GC-FID could make it a very effective tool for acquiring large amounts of data over a long period of time in metabolomics.

T. Sakai, Y. Takemori

Sample Pretreatment and Analysis

Commercially available beer was prepared as a sample, and extraction/derivatization was performed as follows.

After decarbonation by placing it on a sonicator, 50 µL of the beer was collected in a microtube with a capacity of 1.5 mL. After adding 10 µL of 2-isopropyl malic acid solution (0.5 mg/mL) as an internal standard, 250 µL of methanol:water:chloroform = 2.5:1:1 solution was added, followed by shaking for 30 minutes at 37 °C. After centrifuging for 3 minutes at 16,000 G, 225 µL of the supernatant was collected, and 200 µL of ultrapure water was added. After mixing well and centrifuging again for 3 minutes at 16,000 G, 250 µL of the supernatant was collected and dried overnight in a concentrating centrifuge.

80 µL of methoxyamine hydrochloride pyridine solution (20 mg/mL) was added to the dried sample and it was shaken for 90 minutes at 30 °C. Next, 40 µL of N-methyl-N-trimethylsilyl trifluoroacetamide (MSTFA) was added, followed by shaking for 30 minutes at 37 °C. The residue in the solution was precipitated by centrifugation for 3 minutes at 16,000 G, and 100 µL of the supernatant was collected in a GC-MS vial.

The samples were analyzed using GC-FID and GC-MS under the same GC conditions, and the results were compared.

Analysis Conditions

Table 1 shows the analysis conditions. The conditions for GC were in accordance with Smart Metabolites Database™, which is a Shimadzu GC-MS software product.

Table 1 Measurement Conditions

- GC -	
Column	: DB-5 (30 m × 0.25 mm, 1 µm)
Inj. Mode	: Splitless
Inj. Port Temp.	: 250 °C
Oven Program	: (1) 100 °C (4 min) → (10 °C/min) → 320 °C (11 min)
	: (2) 100 °C (4 min) → (4 °C/min) → 320 °C (11 min)
Carrier Gas Flow	: Linear Velocity (39.0 cm/sec)
Purge Flow	: 3.0 mL/min
- FID -	
Temperature	: 330 °C
Sampling Rate	: 40 msec
FID H2 Flow Rate	: 32.0 mL/min
FID Makeup Flow Rate	: 24.0 mL/min
FID Air Flow Rate	: 200.0 mL/min
- MS -	
Analysis Mode	: Scan (*m/z* 35 – 500)
Ion Source Temp.	: 200 °C
Interface Temp.	: 280 °C
Loop Time	: 0.3 sec

Stability after Derivatization in Hours

The chromatogram for GC-FID and total ion chromatogram for GC-MS are shown in Fig. 1. In order to investigate the temporal stability of each peak, we first plotted the transition in intensity over time for the peaks recorded in red (Fig. 2).

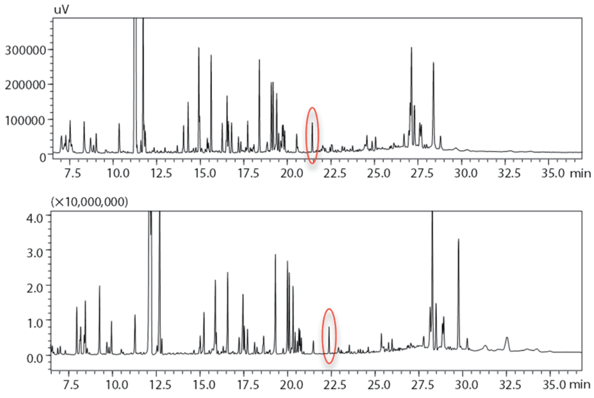

Fig. 1 Chromatogram for GC-FID and Total Ion Chromatogram for GC-MS (Analysis using Temperature Program (1))

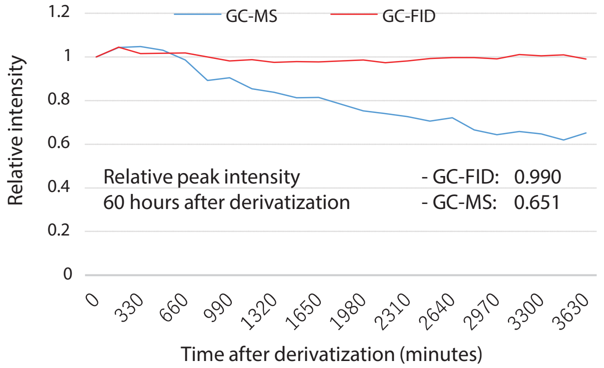

Fig. 2 Transition of the Intensity Value of the Peak Shown in Red in Fig. 1 (Relative value when the intensity value with "time after derivatization = 0" is set as 1)

For the peak shown in red in Fig. 1, with GC-MS the intensity value dropped to 0.65 as a relative value after 60 hours, whereas with GC-FID it was stable at 0.99, meaning it was almost unchanged from 60 hours previously.

Subsequently, as a result of carrying out the same verification for all other peaks, about 80% of the peaks with GC-MS dropped to about 0.65 after 60 hours, whereas with GC-FID about 80% of the peaks remained at around 0.8 to 1.2., so we found there were many peaks that were stable over time (Fig. 3).

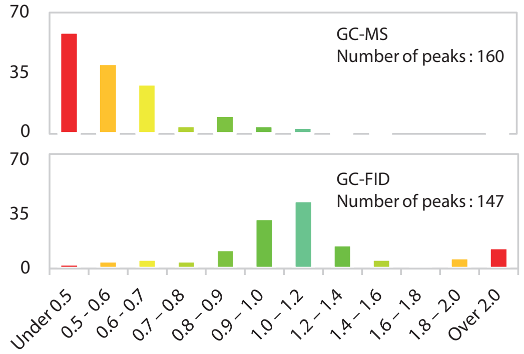

Fig. 3 Distribution of Relative Peak Intensity After 60 hours at all Peaks Detected with GC-MS and GC-FID

Stability in Days

Some of the beer sample pretreated as described above was stored in a dried state, then derivatized and measured 11 days after the measurement on that day, and the results were compared. Fig. 4 shows the chromatograms. With GC-FID, the overall peak height is about the same height as 11 days previously, whereas with GC-MS a tendency to be slightly lower is observed.

Fig. 5 shows a summary of the heights of all peaks. With GC-MS there is a tendency for more than half of the peaks to change in size, whereas with GC-FID it was found that a majority of peaks maintained a value of 0.9 to 1.2.

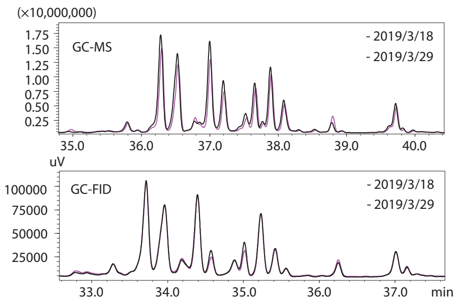

Fig. 4 Chromatograms with GC-FID and GC-MS When the Same Sample Was Derivatized after 11 Days and Analyzed (Analysis using Temperature Program (2))

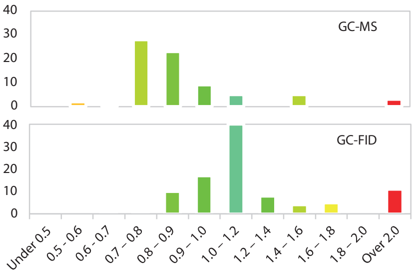

Fig. 5 Distribution of Relative Peak Intensities After 11 Days at all Peaks Detected with GC-MS and GC-FID

Metabolomics Using GC-FID

From among the beers available in the market, five different types that were the same product but made in different factories were prepared, each was pre-treated as described above, and analyzed using GC-FID and GC-MS. Peaks were picked in each set of data, and principal component analysis was performed. The score plots are shown in Fig. 6. It was confirmed that the distributions of the score plots for GC-FID and GC-MS were relatively close to each other.

In metabolomics using GC-FID, it has been shown that other than for qualitative purposes, it may be possible to achieve the same results as with GC-MS, and the results may be better than with GC-MS in terms of data stability.

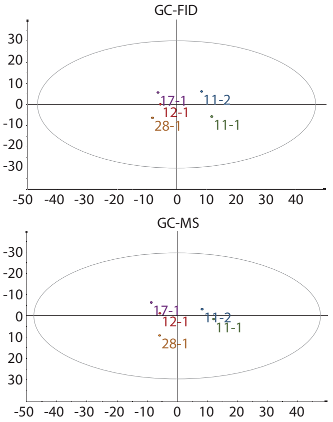

Fig. 6 Score Plots of Five Types of Beer Manufactured at Different Factories (11-1, 11-2, 12-1, 17-1 and 26-1 represent each sample.)

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